Boreal Toad Research in Colorado

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The boreal toad Bufo boreas boreas of the southern Rocky Mountains, has been state-listed as endangered since November 1993, and federally listed as “warranted but precluded” since March 1995. Colorado has four metapopulations which are composed of two or more breeding sites, each with several dozen to several hundred toads. These are 1. Lost Lake/Kettle Tarn in Rocky Mountain National Park, Larimer County, 2. Cottonwood Creek Drainage in the San Isabel National Forest, Chaffee County, 3. Snake River/Ten-Mile Creek, Summit County; and the Clear Creek population in the Arapaho National Forest and the Henderson Mine, in Clear Creek County. Within Colorado, including these metapopulations and a few smaller outlying populations, there are over 50 known breeding localities - some having more than one breeding site. In Wyoming boreal toads were historically found in the Medicine Bow and Sierra Madre Mountains, and the Pole Mountain area of the Laramie Mountains. One breeding population still exists in Albany County, Wyoming. In New Mexico breeding sites were historically located in the Lagunita Mountains in Rio Arriba County, but currently there are no known active breeding sites. A sighting of one adult boreal toad and one boreal toad tadpole in September 1996 gives hope that a breeding population may still exist in New Mexico. Governmental listing of the boreal toad was deemed necessary due to evidence of dramatic declines in abundance and loss of populations from 1975 to 1990. Declines of many other amphibian species have been documented world-wide and some scientists believe these declines may be an early indicator of environmental degradation that may ultimately affect other species including humans.

The boreal toad is Colorado’s only alpine species of toad, and has been reported in montane habitats throughout the state at elevations between 7,000 and 12,000 feet (2,135 and 3,660 m). Distribution is restricted to areas with suitable breeding habitat in spruce-fir forests and alpine meadows. Breeding habitat includes: lakes, marshes, ponds, and bogs with sunny exposures and quiet,

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shallow water. Rarely are boreal toads known to lay eggs in streams. Breeding occurs soon after ice melt. Normally breeding takes place in late May or early June, but has been observed as late as mid-July. Young toads are restricted in distribution and movements by available moist habitat, while adults may move up to several miles to reside in marshes, meadows or forested areas. Up to 90% of an adult toads life is spent in upland terrestrial habitats. Hibernation takes place in hibernacula which may be chambers associated with streams or spring seeps, or more commonly, rodent burrows deep enough to prevent freezing and having soil moisture high enough to prevent desiccation. Adult toads monitored in Colorado hibernate at a temperature of approximately 41E F (5E C). Most toads are in hibernation by early October, but association with the hibernacula may begin in late August.

Research Overview

The Colorado Division of Wildlife (CDOW) is mandated to manage and protect amphibians in Colorado, which includes the recovery of the boreal toad. Because the cause(s) of the declines have not yet been clearly identified, many questions need to be answered before we can be expected to successfully recover the toad to its historic abundance and distribution. Since 1995, a broad range of research has been completed, by numerous members of the Recovery Team, as outlined in the Conservation Plan and Agreement. Highlights of this research include UV- radiation impacts, statewide genetic analyses, heavy metal toxicology, habitat use and movement, early life history ecology, predators, long term population monitoring, immunology, pathology, propagation, and now research on chytrid fungus. This research constitutes great progress toward our understanding of boreal toad biology and the circumstances which resulted in population declines. The goals of our research are:

- To conduct experimental reintroductions and supporting propagation.
- To refine knowledge of boreal toad population size, stability, and movement in relation to use of various habitats and define habitat requirements of various life stages.
- To refine knowledge of factors limiting survival of toads, including the chytrid fungus *Batrachochytrium dendrobatidis.*
- To determine the genetic relationship of boreal toads in the Southern Rocky Mountain population and to define management units and recovery strategies on this basis.

One of the research needs identified by the Boreal Toad Recovery Team is to conduct research related to reintroduction actions which includes determining rearing and propagation techniques and conducting experimental reintroductions. Several introduction efforts have taken place over the last few years but all have failed. Reintroduction and captive propagation and rearing may be a vitally important technique in the future management of the boreal toad. Therefore, it is essential that the methods for reintroduction, propagation, and rearing be investigated and well-defined. From 1994 to 1996 the techniques for captive rearing and breeding of boreal toads were developed at the CDOW Research Hatchery. We have now established a large captive brood population at the Native Species Restoration Facility in Alamosa and are continuing to work out captive breeding protocols.

From 1995 to 1998, research on population size, stability, movement, and habitat use was conducted at the Henderson Mine in Clear Creek County, Colorado. The Henderson Mine boreal toad breeding sites consist of numerous ponds and wetlands in an area which was heavily disturbed due to molybdenum mining and was subsequently reclaimed by the Climax Molybdenum Company. The mine is located west of Empire, Colorado at an elevational range of 10,000 to 10,500 feet. Each of six breeding sites at the mine were monitored weekly and all adult toads tagged with passive integrated transponders (PIT tags) to determine population size, stability, breeding site fidelity, and recruitment. In addition, adult toads were fitted with small (1.8g) radio transmitters in 1997and 1998 and located throughout the year to determine movement patterns and habitat use. Locational information was imported into a GIS where we overlaid slope and habitat coverages for analysis. From May to November of each year, we collected locations on individual toads and analyzed this information in relation to habitat use and availability. We have learned that slope is not a deterrent to toad movement and that boreal toads commonly frequent upland habitats and are not always associated with the relatively flat wetland areas as previously thought. There appears to be great heterogeneity among individuals with respect to habitat preferences. Some individuals we tracked spent most of their time in a single habitat type such as conifer while others moved often and used a variety of habitats. Movement was calculated for each toad weekly on a 3 m2 digital elevation model. The average distance moved per day was 11.83 m. Male toads moved an average of 10.60 m per day and females moved an average of 13.93 m per day. The greatest distance moved by any one individual was number 774, a female which moved 643 m in only 28 days. Due to individual heterogeneity, it could not be shown that average movement

distance by female boreal toads was significantly different than males. Movement patterns by female toads were more variable than males and maximum distances moved were far greater in several cases. Boreal toads at the Urad/Henderson breeding sites were PIT tagged during breeding site monitoring activities from 1995 to 2000. Using PIT tag returns we can estimate the number of males at each site for each year monitored. In all cases, the estimates derived from the model used were nearly the same as the total number handled at each site indicating we had PIT tagged and handled close to the entire breeding population of males each year at each site. The number of female boreal toads in the Henderson/Urad area is difficult to estimate because they were never recaptured again in the same year, and only rarely in subsequent years. There was one female tagged in 1995 that returned in 1996 and one tagged in 1996 that returned in 1997. No females that were tagged at one site ever showed up at a different site. This is good evidence that females do not breed every year. There is also evidence that male:female capture rates are skewed toward male dominance. Based on the 1996 estimates, the male breeding population in the Henderson/Urad metapopulation was approximately 227, 233 in 1997, 306 in 1998, 197 in 1999 and approximately 38 in 2000. Assuming a 50:50 sex ratio, the number of breeding age adults in the population was reduced 36% in 1999 and 88% in 2000 (compared with 1998). It is probable based on pathology results that these reductions are primarily due to chytridiomycosis. In 2000, six of nine mortalities were confirmed positive for chytrid fungus, two were negative, and one was suspect but inconclusive. We will continue to monitor the Henderson population in 2001 to document the outcome of this disease event.

This type of work is critical in defining what is natural fluctuation in breeding numbers over time and identifying declines. Trends in population size and breeding success at all known boreal toad breeding sites are being monitored on an ongoing basis. This information will permit rapid identification of changes in abundance which could influence recovery. It is obvious that not all sites recruit every year and some fluctuation is natural. In most cases, individual breeding sites recruit in one out of three years at best.

In the late 1990s, researchers discovered a chytrid fungus (*Batrachochytrium dendrobatidis*) infecting frogs in areas experiencing amphibian population declines in Central America and Australia. In 1999, a decline in the Henderson/Urad boreal toad population in Colorado was attributed to this “frog chytrid”. Subsequent pathological work has shown that chytrid fungus was present at this locality as early as 1995. Chytrid fungus has now been identified in boreal toads from at least three populations and

evidence exists that this pathogen was in Colorado during the declines in the late 1970's and early 1980's.

The chytrid life cycle begins with a motile zoospore, which is the infective stage of this pathogen. During the course of infection, chytrid zoospores enter skin cells on the amphibian. The fungus grows and develops asexually within the skin cells. Eventually, discharge tubes form that extend to the surface of the cells. Mature zoospores emerge from the discharge tube and begin the life cycle again. Infections are restricted to the skin of the amphibian. Infected amphibians often slough the skin more frequently than healthy amphibians. At this time, it is not known exactly how this fungus kills amphibians. Future research must be aimed at mitigating the adverse impacts of this pathogen on the boreal toad, both in the wild and in captivity.

PCR Sampling TC \l1 "

Currently the only way to positively diagnose chytrid fungus infection on boreal toads is through histological examination of dead toads. We are in the process of having a polymerase chain reaction (PCR) test developed in cooperation with the University of Maine and the USGS Biological Research Division. This test will enable us to identify chytid fungus DNA from a small sample such as a piece of skin from a live toad. We are in the process of collecting samples from all known populations to determine the extent of chytrid fungus in boreal toads statewide.

Twenty-nine *Bufo boreas* breeding sites were visited at least once and often two or more times during the 2000 field season . Four of these sites did not yield any toads, so are not represented by samples. A total of 150 boreal toads were sampled at the 26 sites that produced one or more toads.

Three of the sample areas are known to be positive for chytrid fungus. That is, researchers have submitted one or more toads for pathological examination and the toads were determined to be infected. These areas are the Woods Creek drainage (Clear Creek County), North Fork drainage in Rocky Mountain National Park (Larimer County), and Conundrum Creek (Pitkin County). We obtained one or more samples from each of these areas. In addition to presence or absence of disease, it also is important to determine the prevalence of disease. Ultimately, effective management of boreal toads will require an understanding the other factors, both biotic and abiotic, that may affect the fate of a population affected by chytrid fungus.

Sites were also sampled that are not known to be positive for chytrid fungus, but are geographically proximate to positive areas.

Results from these sites may provide information on chytrid dispersal. Of particular interest is information on what geographic or landscape features may constitute effective barriers to chytrid dispersal.

In addition to sampling boreal toad sites, 64 samples were collected from seven other amphibian species. Of these species, the northern leopard frog (*Rana pipiens*) has also experienced population declines that likely are attributable to chytrid fungus infection. Samples were collected from this species in two areas on City of Boulder Open Space.

In 1999, Woodhouse's toads (*Bufo woodhousii*) in No Thoroughfare Canyon, Colorado National Monument (Mesa County) experienced a die-off. Because of the possibility that chytrid fungus might have been involved in this mortality event, it was important to try to collect samples from this species. Conditions were very dry when No Thoroughfare Canyon was visited, and only one amphibian (a canyon treefrog, *Hyla arenicolor*) was observed. However, samples were obtained from juvenile Woodhouse's toads from an adjacent canyon.

Other amphibian species have not experienced obvious population declines. Whether these species act as reservoirs for chytrid fungus or simply are less susceptible to infection is not known. At low elevations, non-native bullfrogs (*Rana catesbeiana*) have replaced native leopard frogs in many areas. Tiger salamanders (*Ambystoma tigrinum*) and chorus frogs (*Pseudacris triseriata*) occur in the mountains of Colorado, but unlike the boreal toad, have not experienced detectable contractions in geographic distribution. Most of the tiger salamanders were sampled from the Grand Mesa, an area proposed as a potential restoration site for boreal toads.

A limited samples of local amphibians were also collected in the vicinity of the John W. Mumma Native Aquatic Species Restoration Facility in Alamosa. Because this hatchery is the site for rearing of boreal toads, it is important to learn whether chytrid fungus is present in the area.

The sampling efforts described here cannot be analyzed until the completion of the PCR test. However, when complete, the results will provide important information on the distribution of chytrid fungus in Colorado. Future management efforts for the boreal toad will require a better understanding of the chytrid fungus, its ecology, and its effects on amphibian populations.

Toxicology

The CDOW Aquatic Toxicology Laboratory is assisting with

investigations into possible causes of this decline by evaluating water quality characteristics that may limit survival and distribution of boreal toad tadpoles. These efforts include analysis of water samples collected from current and historic breeding ponds, developing techniques to measure effects of toxicants (heavy metals, pesticides, deicing compounds) to tadpoles, and conducting experiments to determine toxicity of selected metals to boreal toad tadpoles.

In various tests performed by the lab, boreal toad tadpoles were much more resistant to the acute lethal effects of cadmium than other aquatic vertebrates. The 96 hour LC50 for boreal toad tadpoles was 582 Fg/L compared to 2.7 and 3.0 Fg/L for rainbow trout, Oncorhynchus mykiss, in water with similar characteristics. The concentrations of cadmium required to kill boreal toad tadpoles are quite high and much greater than would be found in breeding areas in Colorado. However, the sublethal effects observed which include reduced growth occurred at concentrations well below lethal levels. These sublethal effects could be detrimental to survival of tadpoles in their actual environment. For example, reduced growth may result in increased risk of predation and decreased overwinter survival of toadlets. A delay in development could lead to recruitment failure if tadpoles are unable to metamorphose to toadlets prior to onset of winter; a likely possibility given the short summers at the higher elevations where boreal toads tadpoles develop.

The acute toxicity of copper to boreal toad tadpoles is remarkably similar to rainbow trout (Oncorhynchus mykiss). The 96 hr LC50s for boreal toad tadpoles from static renewal and flow-through exposures were 57.1 and 69.4 Fg Cu/L, respectively. Other work at the CDOW Water Quality Lab has indicated 96 hr LC50s of 46.6-55.7 Fg Cu/L to rainbow trout exposed to copper in similar water quality. The copper concentrations that affected growth and survival for boreal toad tadpoles are similar. This contrasts with cadmium where growth and development were affected at cadmium concentrations well below lethal levels.

The results of experiments on manganese toxicity indicate that boreal toad tadpoles are relatively tolerant of this metal. The 96 hour LC50 for rainbow trout in similar water quality was 4.8 mg Mn/L; about ten times lower than tadpoles at 42.3mg Mn/L. However, the duration of exposures for this experiment were relatively short (10 days). The toxicity of manganese increased substantially over time. For example, the LC50 at 168 hours was less than half of the 96 hour LC50. Longer exposures would be required to assess chronic and sublethal effects.

Boreal toad tadpoles were more tolerant of zinc than rainbow

trout. The median lethal concentration for rainbow trout in similar water ranges between 370 and 756 Fg/L . The duration of exposures of this experiment were relatively short (10 days) and longer exposures would be required to assess chronic and sublethal effects.

Tadpole Ecology

Analysis of factors affecting the survival of early life stages has conservation implications, since increased recruitment could enhance the recovery of toad populations. In addition, investigation of habitat characteristics associated with successful survival to metamorphosis, including predator communities, can aid in selection of sites for reintroduction efforts.

Predation on eggs, tadpoles, or metamorphosed toads has not been suggested as a direct cause of population declines in this species in Colorado. However, many formerly large boreal toad populations have been eliminated or reduced to small remnants. With the reduced abundance of boreal toads, natural predation events may be a threat to some remaining toad populations. Three predators readily consumed large numbers of B. boreas tadpoles or toadlets in laboratory trials: predaceous diving beetle (Dytiscus sp.) larvae, Ambystoma tigrinum larvae, and Thamnophis elegans. Various medium-sized adult dytiscid beetles (Agabus tristis, Rhantus binotatus, and Graphoderus occidentalis) were minor tadpole predators. The leech Nephelopsis obscura, backswimmer Notonecta undulata, and caddis fly larva did not consume any tadpoles in the laboratory trials. Efforts to remove and relocate T. elegans and Dytiscus larvae were initiated at some boreal toad breeding sites. These efforts should continue and be expanded to include other sites where these predators may decrease boreal toad tadpole populations. In addition, the presence of Dytiscus larvae, A. tigrinum larvae, and T. elegans should be among the factors considered in evaluating potential boreal toad reintroduction sites.

High densities of newly metamorphosed individuals have been noted for various members of the genus Bufo (boreas, americanus, carens, cognatus, marinus, punctatus, viridis), the ranid Aubria subsigillata, and spadefoot toads (Spea intermontana, Scaphiopus holbrookii). Since the first report of post-metamorphic aggregations in Bufo boreas, such aggregations have been observed throughout the species= range. Several environmental and behavioral factors may influence the formation of post-metamorphic aggregations (PMA): (I) deteriorating larval environment, (ii) inability to disperse, (iii) protection from desiccation, (iv) enhancement of insulation or thermal environment, and (v) selfish herd/predator saturation.

B. boreas in Colorado is a high-elevation form for which the thermal regime may be especially important. This research has indicated that these aggregations of newly metamorphosed toadlets are related to protection from desiccation and there is evidence that there is a thermal advantage associated with larger aggregations, since in most instances, there is a positive and significant correlation between aggregation size and temperature. The active movement by toadlets to aggregations and within aggregations may serve an additional function: minimization of excessive exposure to dangerous levels of UV radiation in an area with scant shade. Under sunny conditions, Bufo boreas metamorphs at this elevation approach their tolerance level to UV-B. There is strong evidence that large and enduring PMAs indicate that newly metamorphosed anurans are unable to disperse.

Genetics

Specimens of Bufo boreas collected throughout Colorado and southeast Wyoming were analyzed using two mitochondrial data sets: 1) sequences of the control region identified by single-stranded conformational polymorphisms and restriction-site polymorphisms, and 2) sequence data from the whole mitochondrial DNA (mtDNA) based on restriction-site polymorphisms. Both methods detect divergent mtDNA haplotypes (also called alleles). Results of analyses suggest:

1. All specimens analyzed in Colorado and southeast Wyoming fall into a cluster of closely related haplotypes.
2. The three largest populations in Colorado (Rocky Mountain National Park, the Clear Creek Drainage, and Chaffee County) all have unique mtDNA haplotypes and significantly different frequencies of shared mtDNA haplotypes. Specimens within the Clear Creek drainage have a pattern consistent with a metapopulation structure (all populations represented by few individuals are subsets of the largest population at Henderson Mine).

Previous analyses of mtDNA of the Bufo boreas species group have been reviewed, because results may impact conservation programs for the Colorado and southeast Wyoming cluster. Relevant findings were:

1. Toads in Colorado and southeast Wyoming are within a previously unrecognized and highly divergent mtDNA clade described here as the Southern Rocky Mountain clade. The mtDNA of toads in Colorado and southeast Wyoming does not form a

monophyletic mtDNA clade.
2. Mitochondrial data suggest that toads originally migrated into Colorado from northern Utah, because haplotypes very closely related to those in Colorado and southeast Wyoming have been found in northern Utah.

All data combined suggest a four tiered approach to conservation management. Toads in the Southern Rocky Mountain mtDNA clade (Colorado, southeast Wyoming, southeast Idaho, and northern Utah) should be managed together as the most inclusive unit and separate from Bufo boreas elsewhere. Toads from northern Utah, Colorado, and southeast Wyoming should be managed with cooperation among states, because toads from these regions appear to be closely related. For example, should toads from Colorado and southeast Wyoming go extinct, northern Utah might provide the best source population for reintroductions. Toads from the geographically disjunct region of Colorado and southeast Wyoming should be managed as a third tier, because populations in these regions have closely related mtDNAs and they are geographically disjunct from toads elsewhere. The smallest management units are represented by three regions within Colorado (Rocky Mountain National Park, the Clear Creek Drainage, and Chaffee County). These populations are differentiated from each other by both unique alleles and significantly different frequencies of shared alleles.

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